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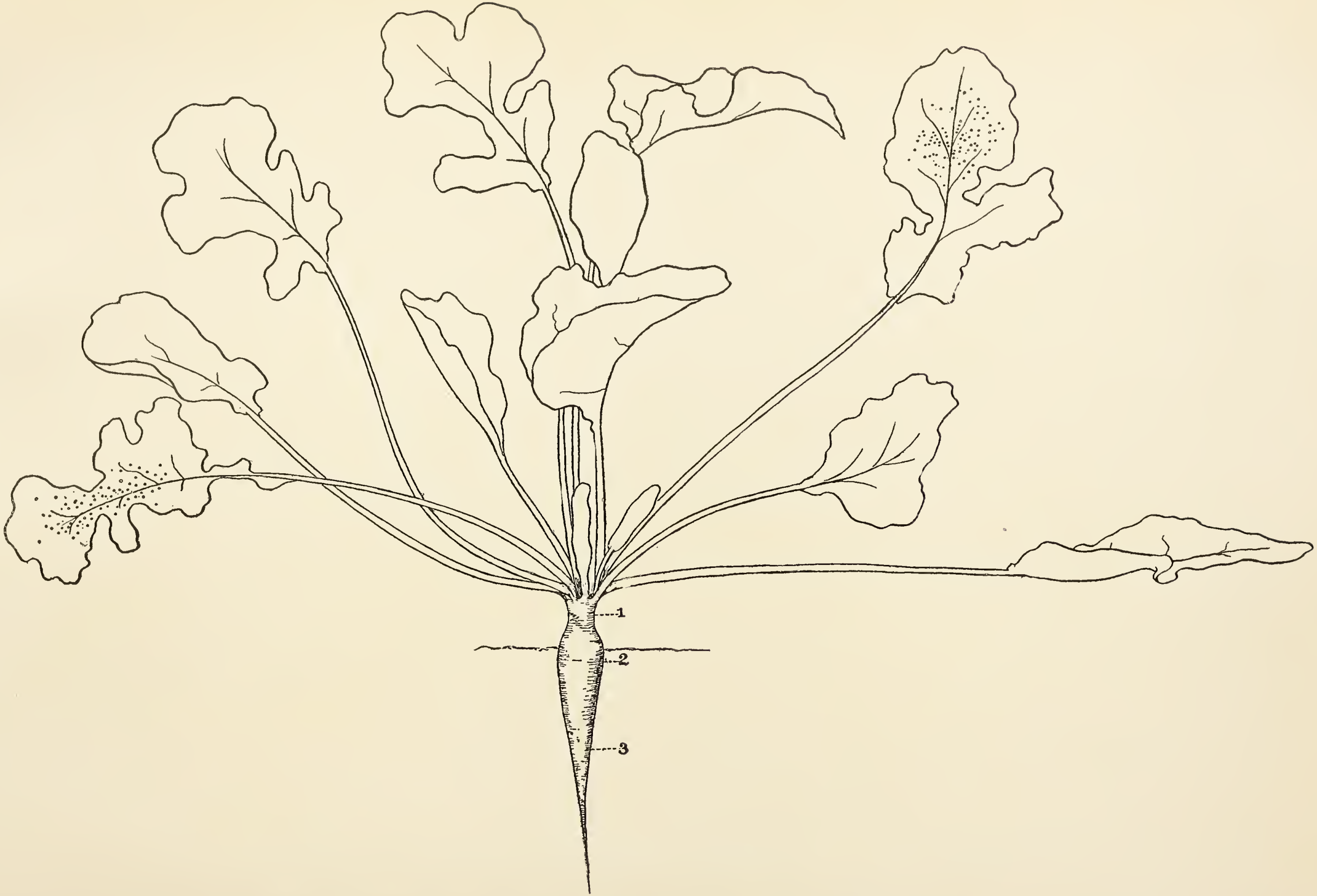

Turnip Plant, Showing Inoculations on Leaves and Indicating the Parts of the Root from which Sections were Taken for the Photomicrographs.

U. S. DEPARTMENT OF AGRICULTURE.
BUREAU OF PLANT INDUSTRY—BULLETIN NO. 29.
B T. GALLOWAY, *Chief of Bureau.*

THE EFFECT OF BLACK ROT ON TURNIPS:

A SERIES OF PHOTOMICROGRAPHS, ACCOMPANIED BY AN EXPLANATORY TEXT.

BY

ERWIN F. SMITH,
Pathologist, Laboratory of Plant Pathology,
VEGETABLE PATHOLOGICAL AND PHYSIOLOGICAL INVESTIGATIONS.

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Plant Industry,
Office of the Chief,
Washington, D. C., October 7, 1902.

Sir: I have the honor to transmit herewith a technical paper on "The Effect of Black Rot on Turnips: A Series of Photomicrographs, Accompanied by an Explanatory Text," and respectfully recommend that it be published as No. 29 of the series of bulletins of this Bureau. The paper was prepared by Dr. Erwin F. Smith, in charge of the Laboratory of Plant Pathology, Vegetable Pathological and Physiological Investigations, and was submitted by the Pathologist and Physiologist.

Respectfully,

B. T. Galloway,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

PREFACE.

Dr. Smith's studies of the bacterial organism causing the black or brown rot of turnips dealt with in this paper were begun in September, 1896. In other papers he has discussed the morphology and cultural peculiarities of the parasite and has pointed out methods for limiting the spread of the disease. In this paper he confines his attention to the action of the parasite on the host plant, demonstrating by means of the microscope and camera the ability of the organism to destroy cell walls, and illustrating various stages in the progress of the disease. The paper is timely in that the ability of bacteria to destroy cell walls in living plants is still disputed in many quarters and is a subject left untreated in most of the text-books.

The paper is technical and is designed for the use of investigators in plant pathology.

Albert F. Woods.
Pathologist and Physiologist.

Office of the Pathologist and Physiologist.
Washington, D. C., October 6, 1902.

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THE EFFECT OF BLACK ROT ON TURNIPS.[a]

INTRODUCTORY

Very few persons would now have the hardihood to deny the existence of plant diseases due to bacteria, but great ignorance still exists respecting this class of diseases, and particularly respecting the capacity of these bacteria for destroying cell walls and making their way, unaided by other organisms, from one part of the plant to another. Even so good a physiologist as H. Marshall Ward, in so recent a book as Disease in Plants (1900), knows nothing about the destruction of cellulose by bacteria, and is inclined to think that, in most cases of "bacterial" disease, fungi act as carriers of the bacteria or forerunners.

The writer has found so much of interest attached to the study of his slides that he is prepared to believe that photographs of them will be of more or less general scientific interest. The behavior of *Pseudomonas campestris* when inoculated into cruciferous plants is not unique, and this particular organism has been selected accidentally, rather than for any special reason, to illustrate what I have to say at this time. The writer possesses alcoholic material of equal interest from many kinds of plants inoculated with various bacteria and a single turnip plant attacked by one organism has been selected for these illustrations, principally because the material proved excellent and appeared to be sufficient for the purpose in hand. The figures will also serve to illustrate how much may be learned from the careful examination of a single specimen.

The following study is purely morphological, and it, of course, raises various questions which can be settled only by the isolation of a cytase. Judging by the time required for cruciferous plants to get into the condition shown in inoculated plant No. 53,[b] the enzymic action on the cell walls must be rather slow, and experimental evidence with the precipitated substances containing the enzyme would probably be less readily obtained than in case of those bacteria which act on the tissues very rapidly. The isolation of the enzyme, and the study of its action were tempting subjects, but the writer's time was very fully occupied with other matters, and the requisite leisure could not be found. This is the less to be regretted, however, since we may confidently expect this phase of the subject to be treated very fully and satisfactorily in the forthcoming papers of Jones and Potter.[a]

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[a] This disease is also called brown rot.

[b] The plant from which sections were cut for nearly all of these illustrations.

GENERAL CONSIDERATIONS.

So far as I can determine from sections, *Ps. campestris* is capable not only of destroying the middle lamella, but also of dissolving the cell wall proper. This it does slowly. At first I thought that I detected a swelling of the walls prior to their disappearance, but subsequent comparative measurements of the walls supposed to be swollen with normal walls left me in doubt. Fresh material, which has not been examined to this end, might give a very different result. That the solution of the cell walls is progressive is shown by the fact that many of the walls still remaining in the bacterial masses are only one-third to one-fourth as thick as the walls of adjacent uninjured cells. In certain cases where the bacterial mass lies up against the wall of a cell on one side and not on the other, there has been a distinct thinning of the wall on the side next to the bacteria. The difficulty of making exact determinations is increased by the considerable variability in thickness of the walls in the normal parts of the plant.

Russow's cellulose test gave very distinct pictures of the gradual solution and final disappearance of the cell wall. The uninjured cell walls gave a blue reaction, those in process of solution stained feebly or not at all. Sections were also stained in carbol fuchsin, picro-nigrosin, iron hæmatoxylin, and Fleming's triple stain. The vessels of the root are distinguished very readily from the other parts of the root by the safranin of the triple stain, which picks out the lignified reticulations. The bacteria are stained well by carbol fuchsin, by iron hæmatoxylin, and by nigrosin.

The closed bacterial cavities in this root vary in size from openings involving only two or three adjacent cells to spaces formed by the destruction of hundreds, even thousands, of cells. They are full of bacteria when not so exposed that the latter have diffused out into the alcohol.

In many cases parenchyma cells are squeezed together from without and the bacteria do not enter them until they are crushed out of all semblance to cells, but in other cases cells which seem to belong to the parenchyma are filled relatively early without being crushed. These are generally nonlignified wood cells bordering on or lying in the vicinity of the vascular bundles. Probably the organisms bore their way through the cell wall in the manner described by Potter, but the writer has never been able to make out clearly any such penetration.

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[a] Since this was written Mr. Potter's paper has been published. He finds that individual bacteria of *Ps. destructans* bore small holes through membranes previously softened by an enzyme, and in this way enter the cells. Such observations are of course enormously complicated by the minuteness and abundance of the bacteria, and one must be on guard against appearances, which are often deceptive, particularly with dry lenses. Potter's statements, however, are positive, and are based on a number of observations with both fresh and fixed stained material, so that for the present, and so far at least as regards the species in question, we may accept his statements as substantially correct. They will probably soon be verified or contradicted by other observers.

The writer will be pleased at any time to show the slides from which these photographs were made to anyone who is interested, and in exceptional cases will mail slides, or material from which sections may be cut, to those who are particularly interested, this especially because all reproductive processes are imperfect, the gelatin prints being inferior to the negatives and the latter to the microscopic image of the sections. Most of the sections were cut and the photographs made early in 1901, but other work got in the way and delayed the completion of the paper. Most of the negatives were, however, exhibited in the form of lantern slides at several places early in 1901, e. g., University of Michigan, Michigan Agricultural College, The Botanical Seminar of Washington, and the substance of the paper was presented before the Society for Plant Morphology and Physiology at its fifth annual meeting in New York, December 30, 1901, where the lantern slides were again exhibited.

The following statements will be made plainer by a brief account of the structure of the turnip root. As may be seen from Pls. II and III, the basal and swollen part of the turnip root consists of a small pith, a large cylinder of xylem, a narrow cambium cylinder, and a phloem cylinder, beyond which is a cylinder of cortical parenchyma surrounded by cork. In other words, the structure is that of many dicotyledonous stems. The upper part of the root has a larger pith and better differentiated medullary rays. The xylem part of the root contains much wood parenchyma, which is not always easily distinguishable from the medullary rays. The only lignified parts are the reticulations in the vessels. These stain a bright pink with safranin and come out quite distinct from the surrounding wood parenchyma in many of the photographs.

PLANT FURNISHING THE CULTURES.

On Pl. I, fig. 1, is shown the cross section of a turnip root, the interior of which has been destroyed by the bacterium of the brown rot, *Pseudomonas campestris* (Pammel) Smith. This root was collected in September, 1896, from a field near Baltimore.

Turnips attacked by brown rot often live for a long time, but the diseased root does not enlarge much radially. This one, like many others observed by the writer, had the form of a carrot root rather than that of a turnip root, although it was several months old. The walls of the cavities in such roots are usually black or brown, and hence the common name. Frequently there is no external indication of the cavity. For a painting in perspective of such a root see Centralblatt für Bakteriologie, 2 Abt., III Bd., Pl. VI, fig. 1.

THE METHOD OF INOCULATION, ETC.

On the frontispiece will be found a diagrammatic representation of a turnip plant inoculated with a pure culture of *Pseudomonas campestris.* This figure is intended to represent plant No. 53, which was inoculated on the blades of two leaves by means of delicate needle punctures. The plant was then some weeks old and about 9 or 10 or possibly 12 inches high. The material used for inoculation consisted of a well clouded, moderately turbid bouillon culture (eleven days old), which had been used for control in thermal-death point experiments, and which was just beginning to throw down a small amount of yellow precipitate. In other words, the culture was still in active growth and in excellent condition for purposes of inoculation. The original source of the organism was the interior of a turnip such as that which furnished the cross section shown on Pl. I, fig. 1. The inoculations were made in the following manner: Selecting two leaves five or six removes from the lowest leaf, some of the germ-laden fluid was first removed from the tube on the end of a sterile platinum loop and placed on the clean surface of the leaf blades, and then from 75 to 100 delicate pricks were made through this fluid into the leaf, by means of a fine-pointed steel needle, which was passed through the flame before and after use on each plant. Finally, a fresh loop of the bacterial liquid was lifted out of the tube and spread over these punctures. The punctures of themselves did not do the plant any serious injury. The inoculated leaves were covered for an hour or two after the punctures by means of clean white paper—i. e., until sunset. This was done partly to avoid insolation and partly to prevent a too rapid evaporation of the fluid from the surface of the leaf. The leaf surfaces were not sterilized before inoculation for three reasons: (1) Because it was desired to have the inoculations made under conditions simulating as nearly as possible those occurring naturally; (2) because numerous experiments had already shown that with a proper selection of plants such as those used for this series of inoculations, needle punctures unaccompanied by bacteria did not lead to disease; (3) because exposure of the delicate leaves to mercuric chloride or other strong germicides for a time sufficient to destroy all surface spores would, probably, in spite of subsequent washings, have left enough poison on the leaves to inhibit the growth of the parasite, if not to seriously injure the plant. The pricked area on each leaf included perhaps 2 to 3 square centimeters.

SYMPTOMS WHICH RESULTED.

The history of this plant, which was examined nearly every day, is as follows:

December 19, 1896.—Plant inoculated.

December 28.—A slight yellowing of part of the pricked areas.

December 30.—Yellowing and wilt over the whole of the pricked area on one leaf and over one-fourth of the pricked area on the other leaf.

January 2, 1897.—A marked progress of the disease on each leaf. The wilt now involves from 5 to 8 square centimeters on each leaf, and has run out to the margin of each leaf near the apex.

January 4.—The wilt now involves from 10 to 15 square centimeters on each leaf.

January 8.—The wilt is still confined to the two inoculated leaves, about 30 square centimeters on each leaf blade being involved. The advancing part of the diseased area is dull green and flabby. The brown veining on these leaves is not nearly so conspicuous as in the cabbage and kale plants which were inoculated at the same time and from the same cultures.

January 16 (28th day).—Up to this time there have been no constitutional symptoms—that is, no leaves have shown symptoms except those which were inoculated.

February 9 (52d day).—This plant is now badly dwarfed and the top is dying. The four large outer leaves which remain have shriveled nearly to their base, and the bundles in the base of the petioles are plainly blackened. One small leaf is now wilting and shows a distinct blackening of its veins. One other small upper leaf is still green and normal in appearance. This leaf is but slightly developed.

The plant was now pulled up and examined. The rootlets were sound. The main root axis, which was about 3 inches long and one-half inch in diameter in the largest part, was smooth, white, and sound externally. The root was now washed and inspected critically. A most careful examination of the surface of the root gave no indication as to the cause of the disease. The root was then cut open crosswise in three places, namely, at the top, in the middle swollen part, and at the base of the swollen part. In the upper cut, which was made about one-eighth inch under the crown of leaves, the bundles were decidedly black, and many were occupied by the bacteria; there were various small bacterial cavities but no large cavity. In both of the other sections of the root there were small cavities here and there, the affected xylem was pale brown, and in the middle part the whole inner tissue seemed to be softening. The bark part of the root was perfectly sound.

TECHNIQUE EMPLOYED IN STUDY OF DISEASED PLANT.

When examined microscopically, the vessels of some of the bundles were found to be full of bacteria. The vessels of other bundles were free, or filled in part. No fungus threads were present. The root had a turnipy smell when cut. Before putting the specimen into alcohol two tubes of potato were inoculated from the interior. Both yielded a prompt and very abundant growth of the same organism with which the plant had been inoculated fifty-two days before, and no other organism appeared in the tubes. The root remained in strong alcohol for more than four years—i. e., until an opportunity was found for making sections. Portions of it were then placed successively in absolute alcohol, alcohol and chloroform, pure chloroform, cold chloroform containing paraffin, warm chloroform with more paraffin, and finally pure melted paraffin. When thoroughly infiltrated with paraffin they were suitably embedded and cut on the Reinhold-Giltay microtome with a very sharp knife. The sections were floated out and cemented to clean glass slides by means of sterile, distilled water containing one-half per cent of gelatin, freshly prepared. Mild heat was used in straightening out the wrinkles in the sections and the excess of water was removed by setting the slides on end. When dry, the slides were gently warmed until the paraffin was melted, and were then placed in turpentine or xylene until the paraffin had been removed. They were then passed in succession, gently, into Coplin staining jars containing a mixture of turpentine or xylene and absolute alcohol, pure alcohol, graded mixtures of alcohol and water, and finally Ziehl's carbol fuchsin, in which they were allowed to remain from three to five minutes. The excess of stain was removed by leaving the slides in a mixture of equal parts of alcohol and water until the proper differentiation in color had been secured. They were then passed rapidly through graded alcohols into absolute alcohol, and from that into a mixture of alcohol and xylene, into pure xylene, and finally into Canada balsam or Dammar balsam dissolved in xylene. In a very few of the sections here shown, viz, Pl. I, fig. 2, and Pls. II and IV, nigrosin was substituted for carbol fuchsin. A series of sections (cross and longitudinal) from this root were prepared, stained, and studied, and the results obtained are illustrated by means of the accompanying photomicrographs.

The fixing of the root in strong alcohol was considered necessary in order to prevent the bacteria from diffusing out into the fluid. Even 95 per cent alcohol does not entirely prevent diffusion in case the bacterial cavities are large, but does so quite satisfactorily in case of single vessels or small cavities. The knife used for making the sections was very sharp, and did not shove or tear them to any extent, but the strong alcohol in which the material was fixed did, of course, cause more or less twisting and shrinking of the delicate parenchymatous tissues. During the process of hydrating, staining, bleaching, dehydrating, and mounting the cell walls were also occasionally broken and displaced. Observations on the fresh root left no doubt that the vessels were the primary seat of the disease, but the exact limits of the bacterial occupation remained to be determined from properly infiltrated material. This has now been accomplished. The manner of fixing, of infiltrating, and of cutting, and the subsequent fastening to the slide and careful manipulation during the removal of the paraffin, the hydrating, staining, differential bleaching, dehydrating, and mounting processes leaves no doubt whatever that the location of the bacteria in the tissues, as shown in the photomicrographs, is the same as in the fresh root. There has been no tearing of these bacterial masses or shoving or crowding of them into parts of the root where they were not originally present, such as would naturally occur in making sections of living or uninfiltrated material. In some cases the more delicate parts of the root have been broken a little in places, as already mentioned, but only to a slight extent, which in no way interferes with one's judgment as to the effect of the bacteria upon the root. Indeed the process of fixing renders the bacterial layer tougher and less liable to shoving or rupture by the knife than any other part of the root, as may be readily seen from an inspection of my sections. The bacteria in the sections have taken a deep purple stain, and, there being very little ground stain, the individual rods stand out clearly under the oil-immersion lens, much more clearly than in the photomicrographs. The sections are remarkably good, but owing to their thickness (6 μ to 10 μ) a number of layers of bacteria lie one behind the other and seriously interfere with the photographic image. Very likely also a more expert photomicrographer would be able to make more out of the sections than the writer. Attempts were made to cut thinner sections, but the material did not seem well adapted to very thin sections. In those 2–4 μ thick there was so much shoving together and tearing that they could not be used.

SPECIAL ACCOUNT OF THE DISEASED PLANT.

On Pl. II may be seen a cross section of the lower part of the root, magnified 50 times. The section was taken from the point marked "3" on the frontispiece. The bleaching process subsequent to the staining has been carried so far that the root is only slightly stained, except where there is bacterial occupation of the vessels and wood parenchyma. The actual size of this section is indicated by a circle at the bottom of the plate.

The extent of bacterial occupation and disorganization in the interior of the root is very interesting. In one of the cross sections of this root, made higher up than that shown on Pl. II and involving less than one-half the circumference, the writer counted 93 distinct centers of bacterial infection and 15 bacterial cavities, involving in cross section from 50 to 300 cells each. In a cross section from nearly the same level as Pl. II, 146 distinct groups of bacteria were counted in the vessels. In the photograph here reproduced it will be observed that the bacteria are confined to the inner portions of the root, principally to the vessels and the surrounding nonlignified wood parenchyma.

There are no bacterial pockets in the bark part of the root (phloëm and cortical parenchyma), forming the outer 30 to 60 rows of cells. The bacterial foci, of which there are about 130 in this section, are also for the most part at a considerable distance from the center of the root—i. e., in the outer part of the xylem, which, consequently, we may assume either to have been the first portion of the root to become infected or else to have been that part most readily attacked by the organism. The cavities are all or nearly all on the rim of the xylem. There are many infected bundles farther inward in the xylem, but cavities are wanting in that part of the root and are still very small in the outer xylem at this level, and are not clearly visible with this magnification. Farther up the root (Pl. III) the cavities are larger, and only the smaller and medium-sized ones have retained their bacterial contents intact. The sections agree, however, in that the outer part of the xylem has suffered most, and in that all parts external to the cambium are free from infection.

Fig. 2 of Pl. I shows a single small vessel magnified 2,000 times. It is from the inner part of the root and is filled with the bacteria. It corresponds to one of the vessels of Pl. II.

Pl. III is from a cross section in the middle swollen part of the root. (Frontispiece, at point marked "2.") In comparing it with Pl. II, it should be remembered that the magnification is not the same. The actual size of the section is shown on the lower left-hand side of the plate. From the largest cavities the bacteria have diffused out into the alcohol, and only the borders of these cavities are still occupied by the organism. That these open places are also true bacterial cavities and not due to anything else is shown by an inspection of the serial sections, an open cavity in one giving place to a bacterially filled cavity in another, and all being free from fungi and insect injuries. Two of the largest of these full cavities may be seen in the upper part of the picture. On the lower left-hand side of this section is an irregular oblong cavity, which was made by a platinum loop thrust into the tissue to remove some of the organism for cultural purposes.

Pl. IV, fig. 1, represents a cross section taken from the top of the root (frontispiece, point marked "1"), showing transition into stem. The magnification is too small and makes evident much less than the section, but suffices for orientation, and shows that there is no surface wound or large cavity. The pith and the cortical parenchyma are entirely free from the bacteria. Most of the phloëm is also free, but bacteria are present in it at the point marked "Y." Fig. 2 is a detail from the same slide, more highly magnified.

A study of Pls. II, III, and IV, and of corresponding longitudinal sections not here represented, shows that while the vascular system of the plant is very badly infested, the bulk of the tissue is still free from the bacteria; i. e., at least nine-tenths of it, including all of the outer portion of the root which has come into direct contact with the fungi and bacteria of the soil, and which would be more or less rotted if the organism had entered the plant from the earth. If the infection had been from the soil, the root would also have contained a mixture of things and not one species in pure culture, and certainly not *Ps. campestris*, since the soil was not obtained from cabbage fields, and all the numerous uninoculated turnip plants grown in it remained entirely free from this disease.

The following photomicrographs are all made from sections of this root at level No. 2. (See frontispiece and Pl. III.)

Pl. V, fig. 1, represents a longitudinal section through a small bundle fully occupied by the bacteria and deeply stained. The magnification is not sufficient to show the individual organisms, but it can be made out quite clearly that the surrounding parenchyma is not occupied and that there is as yet no disorganization of the tissues. The second figure on this plate is a longitudinal section through two small vascular bundles. The knife passed through the middle of the lower bundle and the extreme margin of the upper one. The tissue around the upper bundle is just becoming hollowed out into a cavity; that around the lower one is still intact and unoccupied by the bacteria. The vessels themselves are crowded full of the organism.

Three of the reproductions on Pl. VI are from cross sections of small bundles, i. e., bundles similar to those illustrated in Pl. V. In the lower right-hand corner is a cross section showing a single vessel occupied by the bacteria, the rest of the tissue being entirely free. In the lower left-hand corner are several vessels so occupied; the larger one, however, contains only a narrow film of bacteria (around the walls), which may have entered from above or below, or at this level, through the side of the vessel next to the more fully occupied part of the bundle. The surrounding tissue here is also entirely free from these organisms. The upper figure on this plate corresponds more nearly to the lower figure on Pl. V. Here several vessels are occupied, together with their connective tissue and the surrounding parenchyma, and we have the first stage in the formation of a bacterial cavity. The left-hand side of this figure is shown more highly magnified on Pl. IX, fig. 1. The middle figures at the left show the manner in which the bacteria crowd apart parenchymatous cells, multiplying first in the intercellular spaces and either dissolving or splitting apart the middle lamella, probably both. The middle figure at the right is a cover glass (smear) of *Ps. campestris* stained with carbol fuchsin and magnified 1,000. It was made directly from the vessels of a cabbage plant and is the only figure not taken from the turnip.

On Pl. VII are two additional figures showing early stages in the occupation of small bundles. Nonlignified wood-parenchyma cells are also occupied, at least in fig. 2. The surrounding tissues are entirely free from bacteria. In both figures the bacterial contents of vessels have fused. This I take to have occurred through natural openings rather than through openings made by the bacteria, because occasionally in cross sections of unoccupied bundles I find reticulations thinning out and disappearing in the same way. In the lower figure a few of the cell walls have been broken and displaced slightly in mounting.

The upper figure on Pl. VIII shows an early stage in the destruction of the parenchyma, the intercellular spaces are occupied by the bacteria, but the cells themselves are still free and have not been wedged apart, as in figs. 4 and 5, Pl. VI. The granular matter in the center of the cells is protoplasmic material. The lower figure shows in cross section a very small vascular bundle. The two reticulated vessels are filled and the bacteria have found their way outside of these into the intercellular spaces which are filled and greatly enlarged, the cells being crowded apart and, in case of one of the lower ones, crushed in. Two or three nonlignified cells are also filled.

Pl. IX shows two stages in the formation of bacterial cavities. In the upper figure there is more or less vagueness in the cell walls, one of which, on the right-hand side, has almost entirely disappeared. This figure shows a single vessel in the center (comparable to fig. 1, Pl. V). The surrounding cells which are so fully occupied by the bacteria are nonlignified wood cells of the fleshy root. In the lower figure there has been a greater softening of tissue and a considerable cavity is in process of formation. Here in the center (from right to left) are at least four vessels, and there is probably a fifth vessel just above the cell marked X. The reader's attention is called particularly to the vagueness of the cell walls and to the open cavity which is shown in the lower middle part and the left-hand part. The whole forms a very instructive and typical picture of the way in which cavities are brought about by the mechanical and solvent action of this organism. The tissue for a long distance around both of these foci is sound and entirely free from bacteria, and, as in the other cases, the bacteria could have entered these bundles only as a result of the leaf inoculations already described.

On Pl. X are shown two figures illustrating a further advance in the destruction of the parenchyma of the root and the formation of bacterial pockets. The cells are separated from each other by masses of bacteria, are crushed in, and are slowly dissolving, their walls becoming vaguer and vaguer until at the middle of the cavity they can not be seen at all. Fine examples of this gradual destruction of the cell walls are to be seen in both of these figures. The reader's attention is called in particular to the right-hand side of the upper figure, showing bacterial occupation of the intercellular spaces which usually precedes the formation of an open cavity. In the upper part of the upper figure, in the last one of the second row of cells at the left, is a fine example of the way the cells are crushed together and loosened from their surroundings. Similar examples may be seen at the bottom of this figure, and in fig. 2. In fig. 2 observe particularly the condition of the cells next above the unoccupied cells marked X, Y, and Z.

The two figures shown in Pl. XI are from the margin of bacterial cavities similar to those shown on Pl. X, but more highly magnified. Here also the cell walls are in all stages of separation and decomposition. In the middle of fig. 1, and at the left side of fig. 2, cell walls may be seen in all stages of solution. In none of these serial sections is there to be found any trace of fungus threads or of insect or other animal devastation, the entire injury being due to an enormous multiplication of the organism which was inoculated into the leaves of the plant, and which found its way into the root through the vascular bundles of the petioles. Part of the injury is plainly mechanical, due to crowding, and part is chemical, due undoubtedly to the solvent action of a cytase. Had the plant been allowed to remain in the soil a few weeks longer, the result must have been the fusing of these various small cavities into one or more large cavities. We should then have had a phenomenon like that shown on Pl. I, fig. 1.

Pl. XII, fig. 1, is from a radial longitudinal section showing cambium and phloem at the right (top) and xylem (wood parenchyma) at the left with cavities close to the cambium. Fig. 2 is a detail from the larger of these cavities taken at the point marked X.

Pl. XIII is a continuation of XII, showing details from the larger cavity taken at the points marked Y and Z. Here again cell walls are crushed and undergoing solutions, and the bacteria are present in incalculable numbers and no other organisms are present. In the upper part of fig. 1, at the right, the bacteria may be seen wedging apart two parenchyma cells. Similar phenomena may be seen in the upper right-hand corner of fig. 2.

RESULTS OF SYNCHRONOUS INOCULATIONS INTO OTHER PLANTS.

At the same time and from the same culture as turnip No. 53 twenty-eight other plants were inoculated as follows: Four rape, 6 radish, 6 cabbage, 5 kale, 3 turnip, and four Roman hyacinth. Twenty-three of the 28 plants contracted the disease, as follows: Four rape—1 plant became diseased constitutionally, 3 showed only local symptoms; 5 radish—3 developed constitutional symptoms, 2 local symptoms only; 6 cabbage—all 6 developed constitutional symptons, and No. 42 was illustrated in 1897 in 2te Abt., Centralblatt f. Bakt. (III Bd., Taf. VI, fig. 5), and in the same journal in 1901 (VII Bd., Taf. VIII and IX); 5 kale—3 developed constitutional symptoms, 2 only local symptoms; 3 turnips—1 developed only local symptoms, the others showed also constitutional symptoms. The hyacinths did not become diseased.

DESCRIPTION OF PLATES.

Frontispiece.—Turnip plant No. 53, showing place of inoculation (on leaves) and indicating by figures the part of root from which sections were taken for the photomicrographs. Diagrammatic. The illustrations were all made from the root of plant No. 53, except fig. 1, Pl. I, from another turnip, and fig. 6, Pl. VI, which was made from a cabbage plant attacked by the same disease.

Plate I. Fig. 1.—Cross section of turnip plant, showing bacterial cavity. Natural infection. From a field near Baltimore. Zeiss planar. x 4½ circa. Fig. 2.—Single vessel from Pl. II, showing bacterial occupation. Stained with nigrosin. x 2000.

II. Cross section of lower part of the root (Frontispiece, 3), differentially stained with safranin and picro-nigrosin to bring out location of the bacteria. x 50.

III. Cross section of a part of the middle portion of the root (Frontispiece, 2), differentially stained with carbol fuchsin, showing numerous bacterial cavities. x 15.

IV. Fig. 1.—Cross section, extreme upper portion of the root (Frontispiece at point marked 1), differentially stained. Zeiss, 70 mm. x 23. Fig. 2.—Detail from the same section (at X) more highly magnified. Zeiss, 16 mm. x 185.

V. Fig. 1.—Longitudinal section in middle of root (Frontispiece, 2) showing reticulated vessels filled with the bacteria and surrounding tissue free from infection. x 154. Fig. 2.—Longitudinal section from same level as Fig. 1, showing vessels filled with the *Pseudomonas*, and a cavity around the upper bundle. x 136.

VI. Fig. 1.—Cross section, middle of root, differentially stained, showing a single vessel packed with the bacteria. This may be compared with fig. 1, Pl. V. x 520. Fig. 2.—Cross section from same portion of the root differentially stained, showing a small bundle partly occupied by the *Pseudomonas*. The larger vessel contains only a rim of bacteria. The surrounding tissue is free from bacteria. x 460. Fig. 3.—Cross section, middle of root, differentially stained, showing vessels and non-lignified wood parenchyma occupied by the bacteria. In the middle portion the cells have begun to disorganize and a bacterial cavity is in process of formation. x 385. Figs. 4 and 5.—Bacteria separating cell walls. x 1000. Fig. 6.—*Ps. campestris.* Smear preparation from a cabbage stem, stained with carbol fuchsin. x 1000.

VII. Figs. 1 and 2.—Cross sections, middle of root, differentially stained, showing small bundles very fully occupied by the *Pseudomonas*. Fig. 1 x 475. Fig. 2 x 470.

VIII. Fig. 1.—Cross section, middle of root, differentially stained, showing parenchyma cells with bacteria multiplying in their intercellular spaces. The cell cavities are free. x 1000. Fig. 2.—Cross section, middle of root, differentially stained, showing disorganization of a small bundle. x 1000.

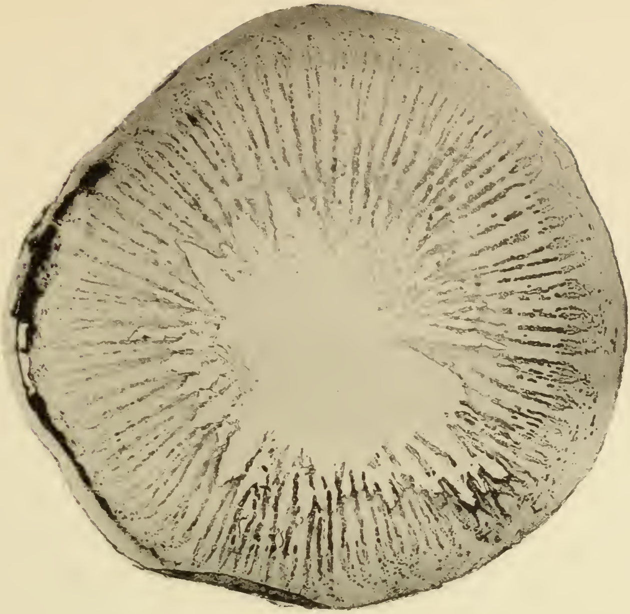
IX. Figs. 1 and 2.—Cross sections, middle of root, differentially stained, showing formation of bacterial cavities. Zeiss apochromatic 3 mm. oil immersion objective 1.40 N. A. and compensating ocular No. 4. Cramer's slow isochromatic plates, Zettnow's color screen, with sunlight. x 1000.

X. Figs. 1 and 2.—Cross sections, middle of root, differentially stained, showing well developed, small cavities filled with the *Pseudomonas* and with remnants of the disorganized cells, the latter conspicuous only on the margins of the cavity. x 340.

XI. Figs. 1 and 2.—Cross sections, middle of root, differentially stained, showing margins of cavities with cell walls in all stages of solution. x 1000.

XII. Fig. 1.—Radial longitudinal section, middle of root, differentially stained, showing one large cavity and several small ones. Bark part of root at right-hand side. x 85. Fig. 2.—Detail from upper left-hand corner (X) of fig. 1. x 475.

XIII. Details from fig. 1, Pl. XII, showing various stages in the separation and solution of cell walls. Fig. 1 is from XII, 1, Y. Fig. 2 is from XII, 1, Z. x 475.



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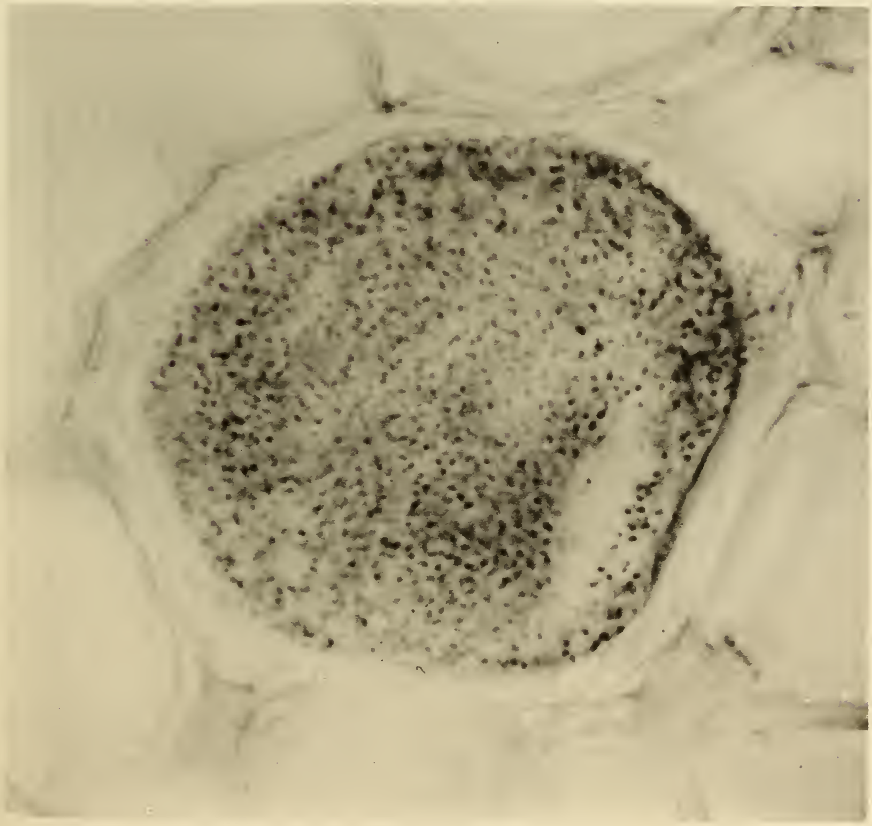

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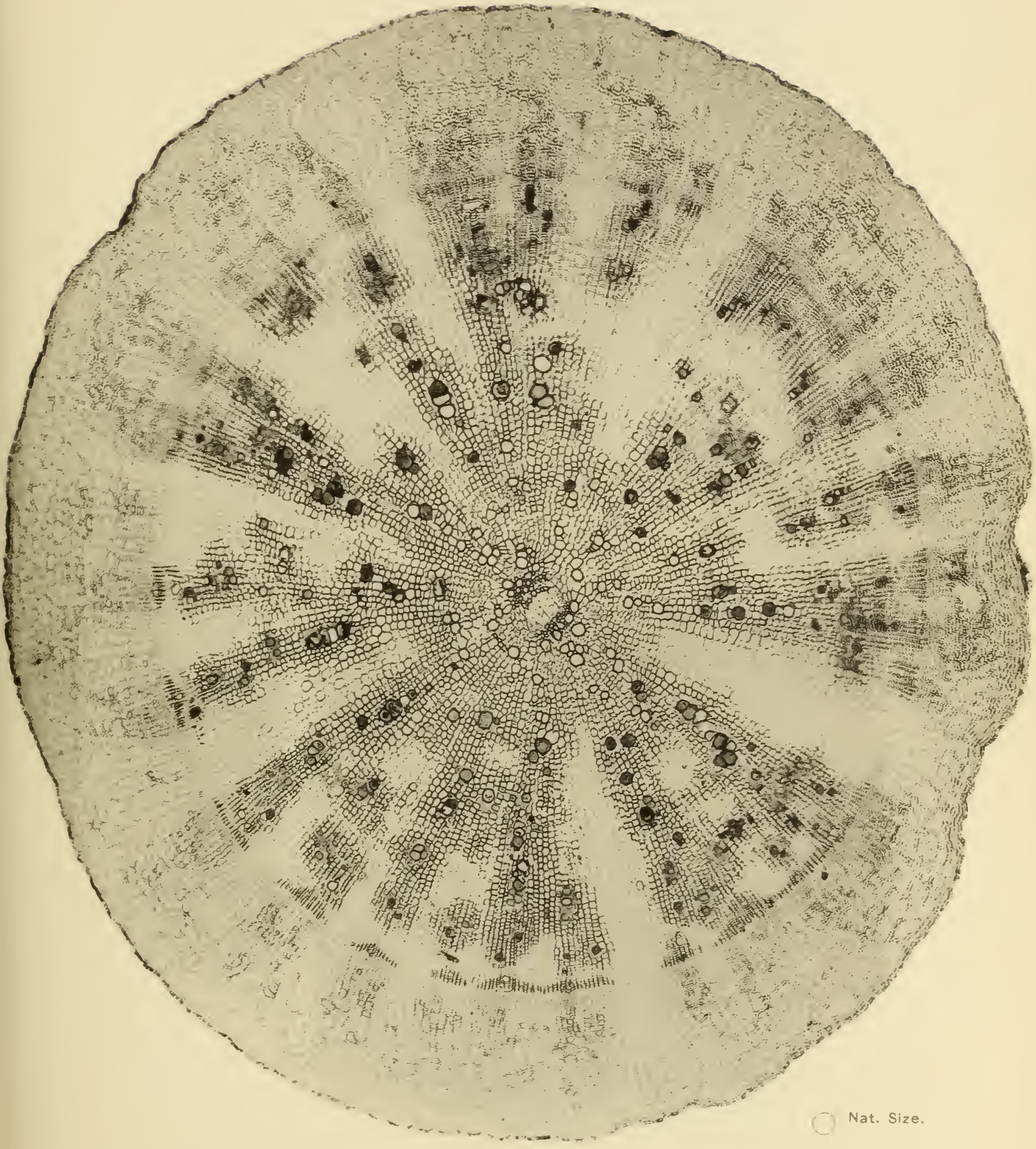
BROWN ROT OF THE TURNIP.

PSEUDOMONAS CAMPESTRIS (PAMMEL) SMITH.

HELIOTYPE CO., BOSTON.



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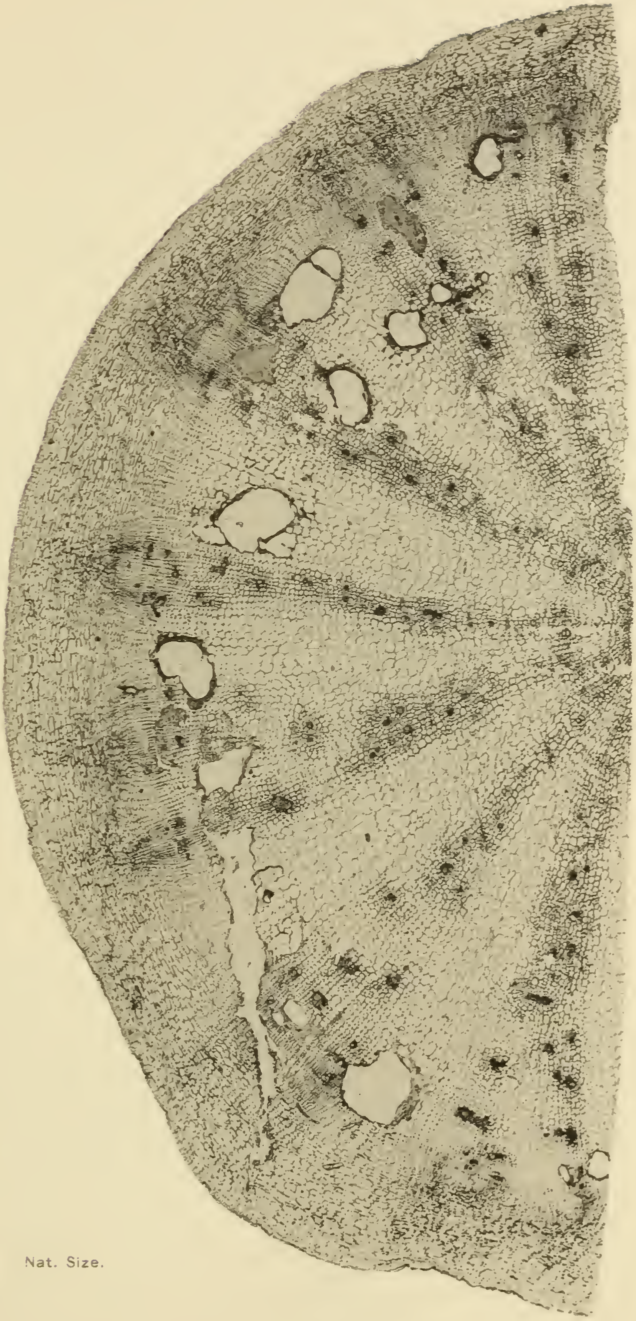

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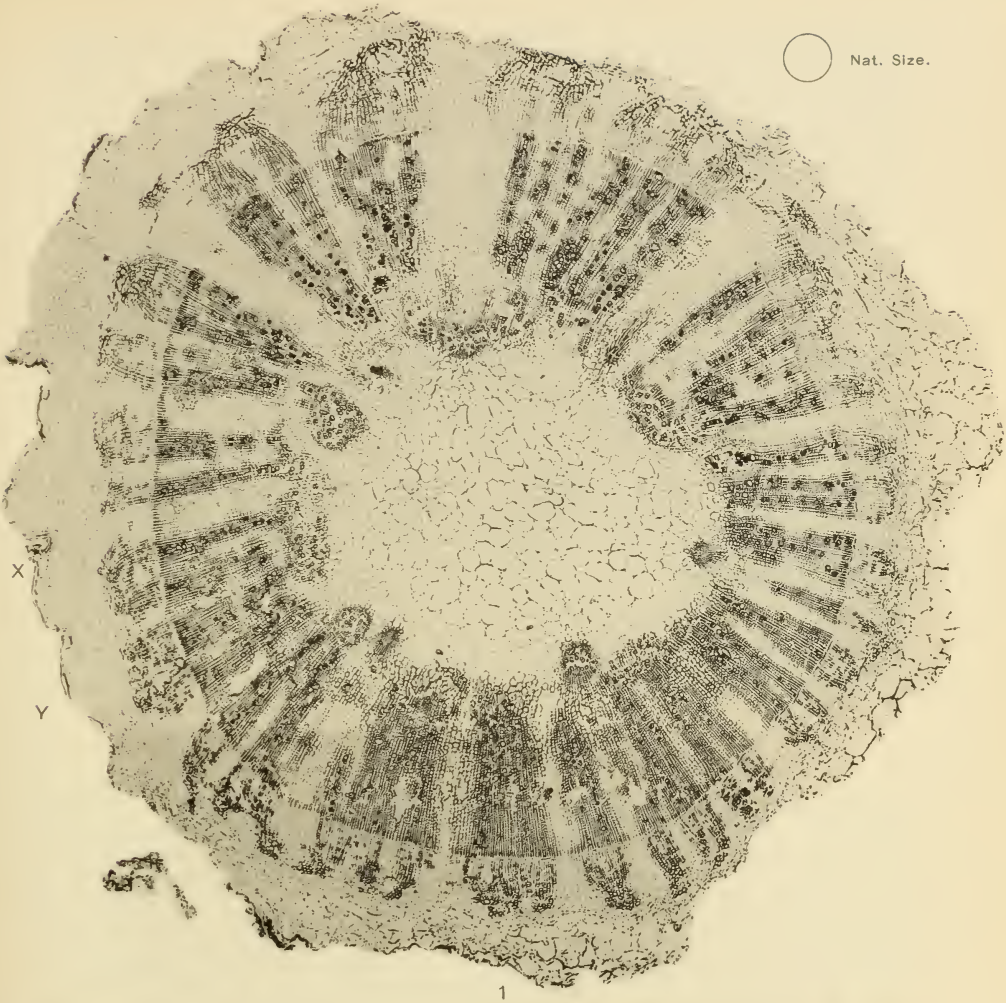

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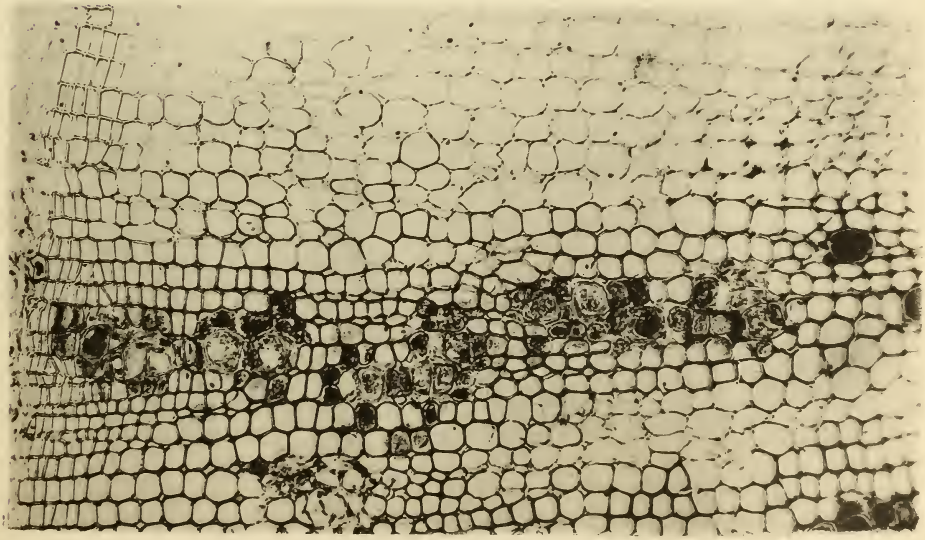

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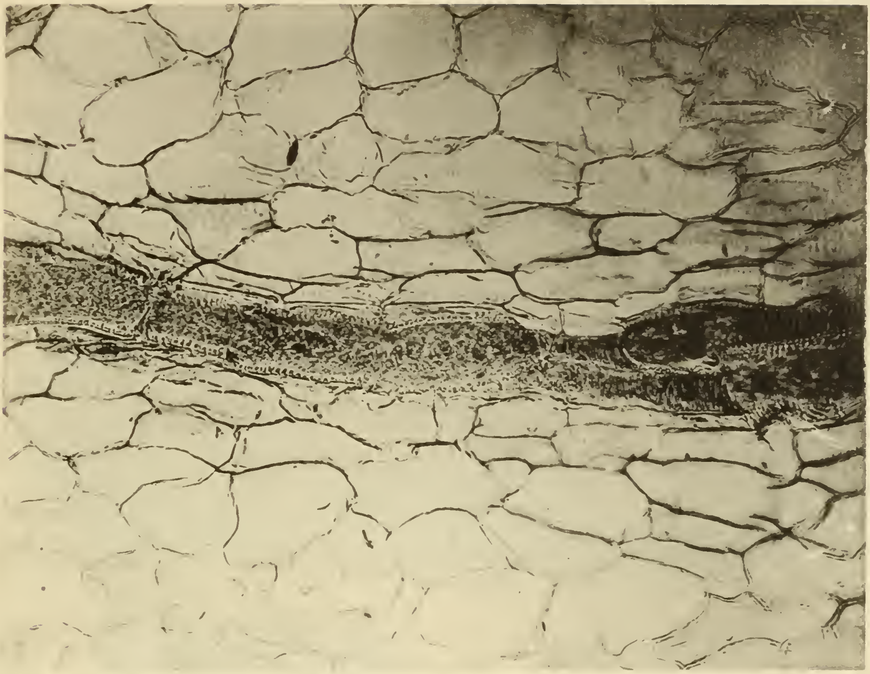

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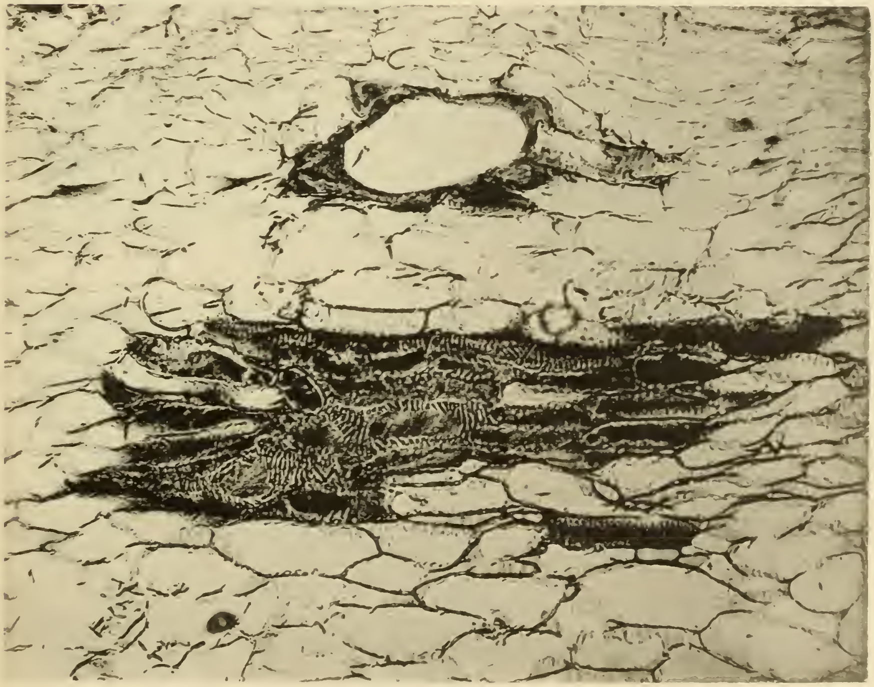

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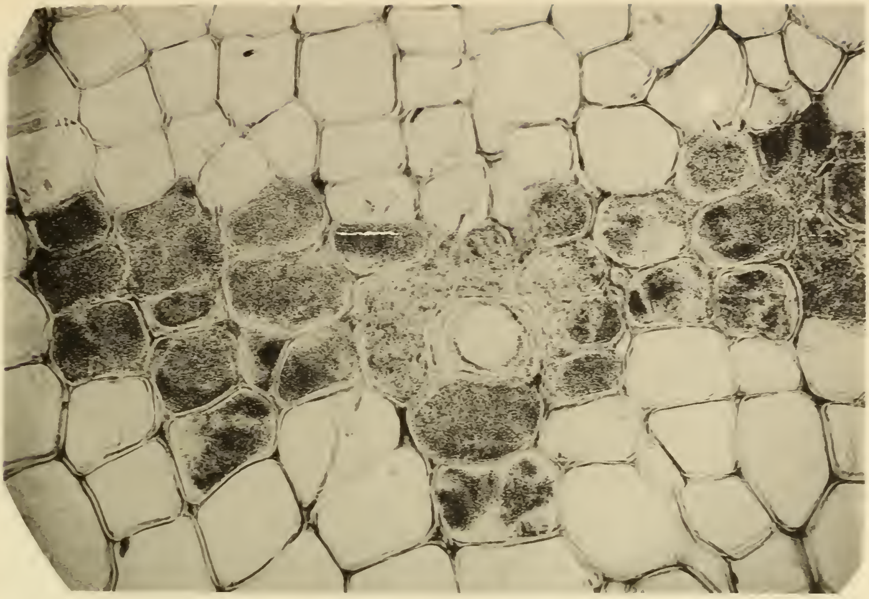

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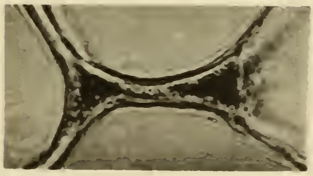

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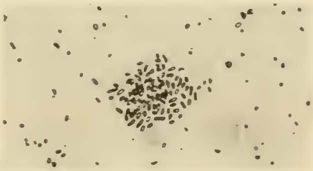

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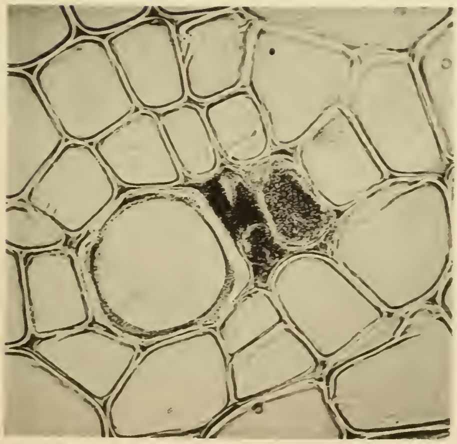

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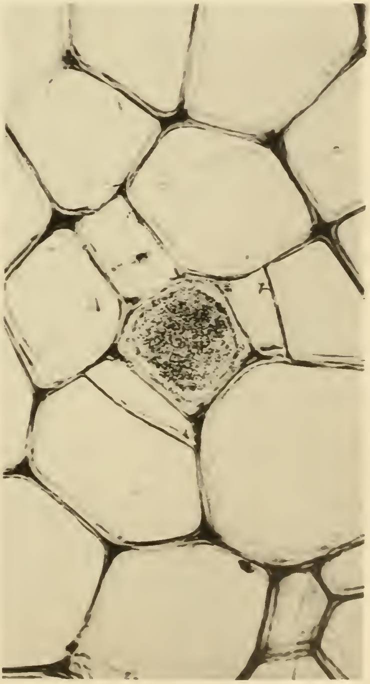

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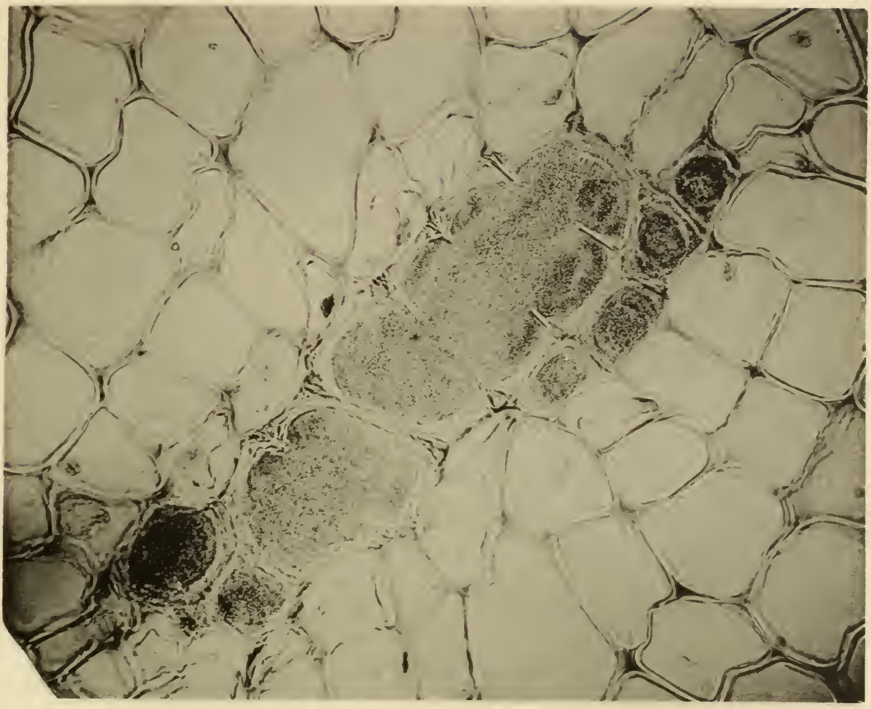

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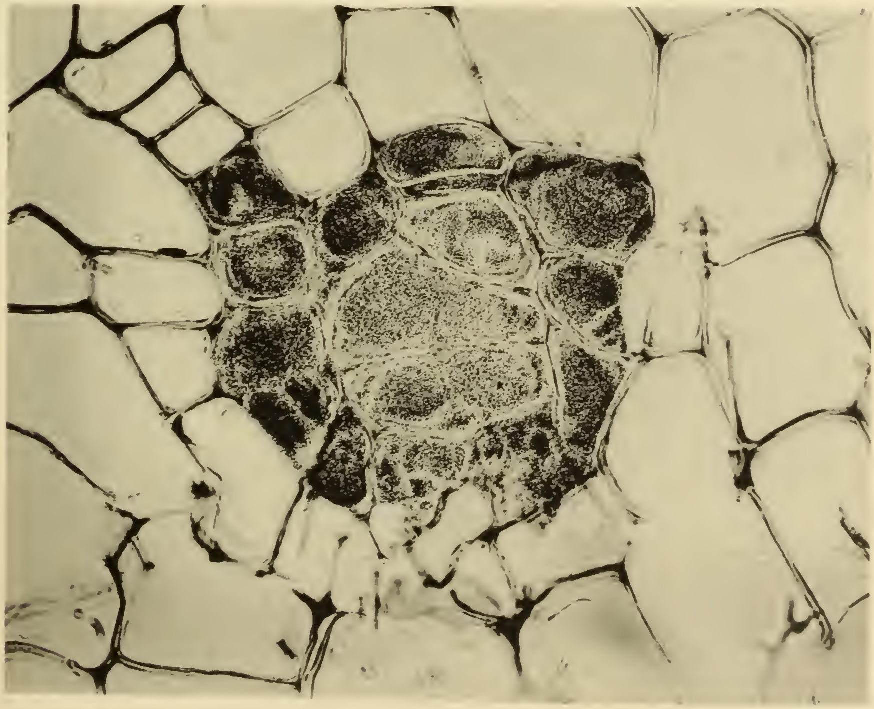

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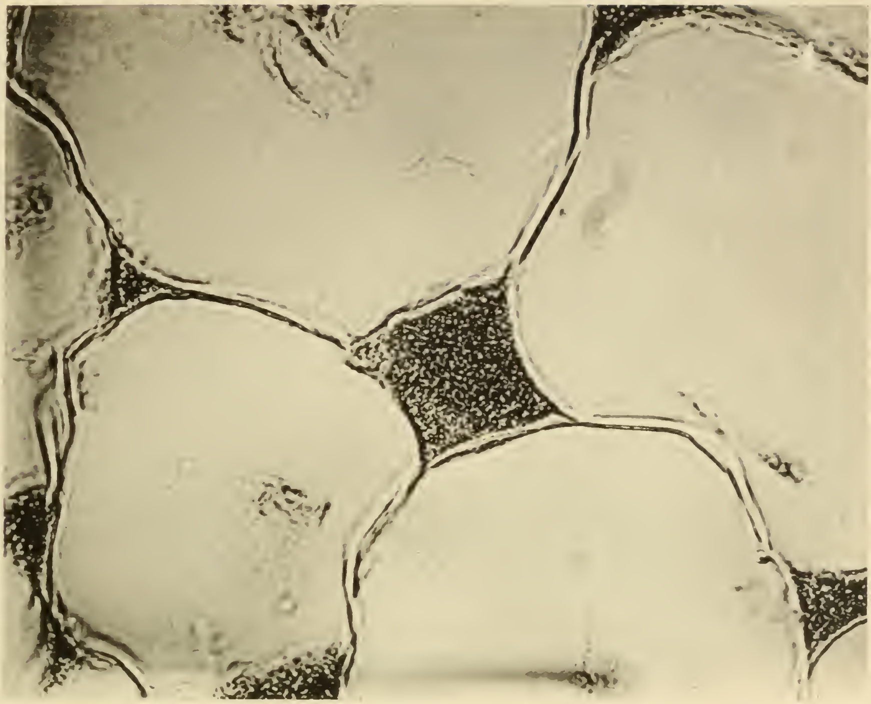

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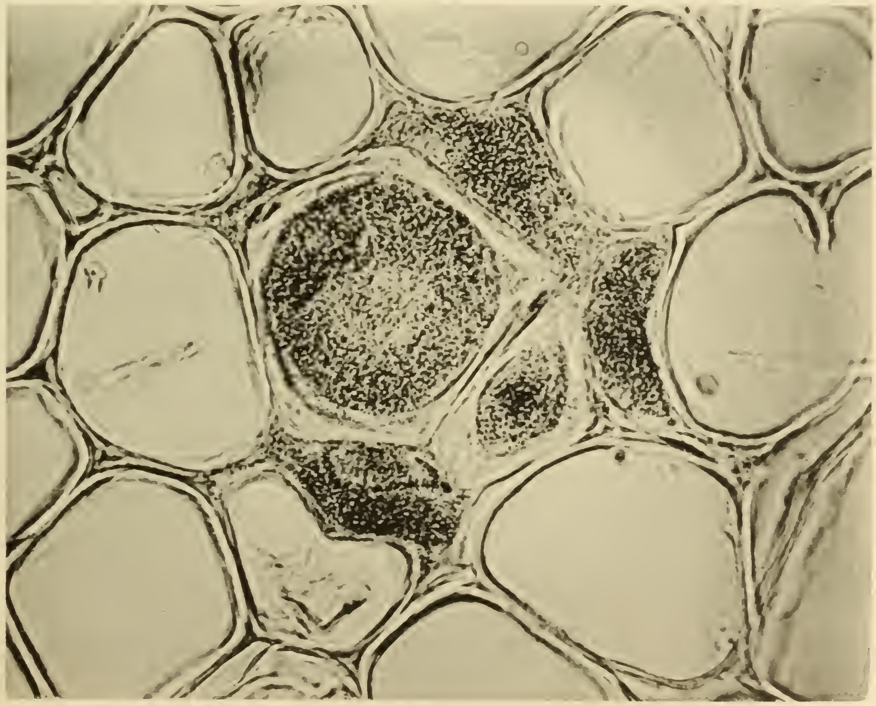

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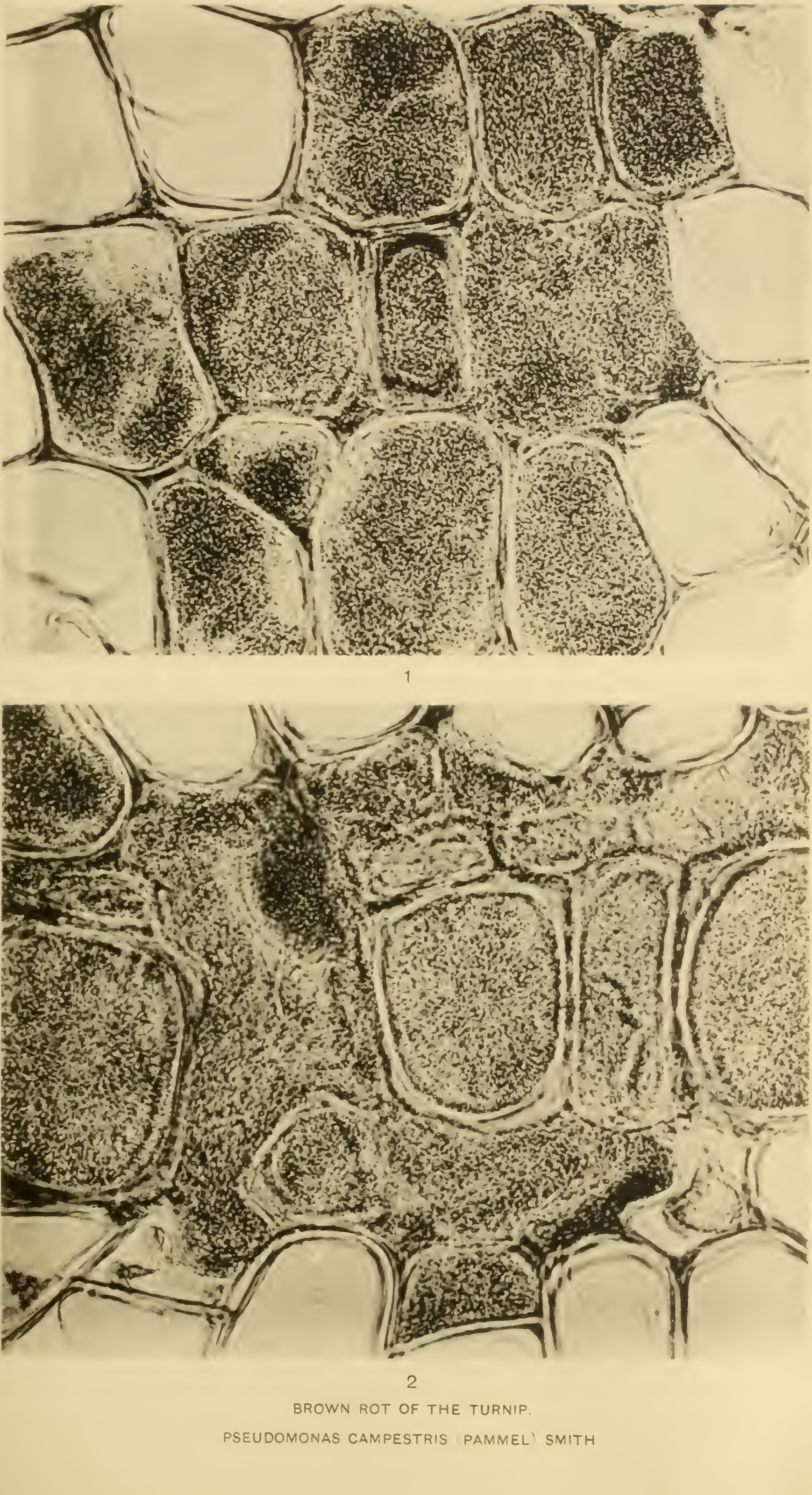

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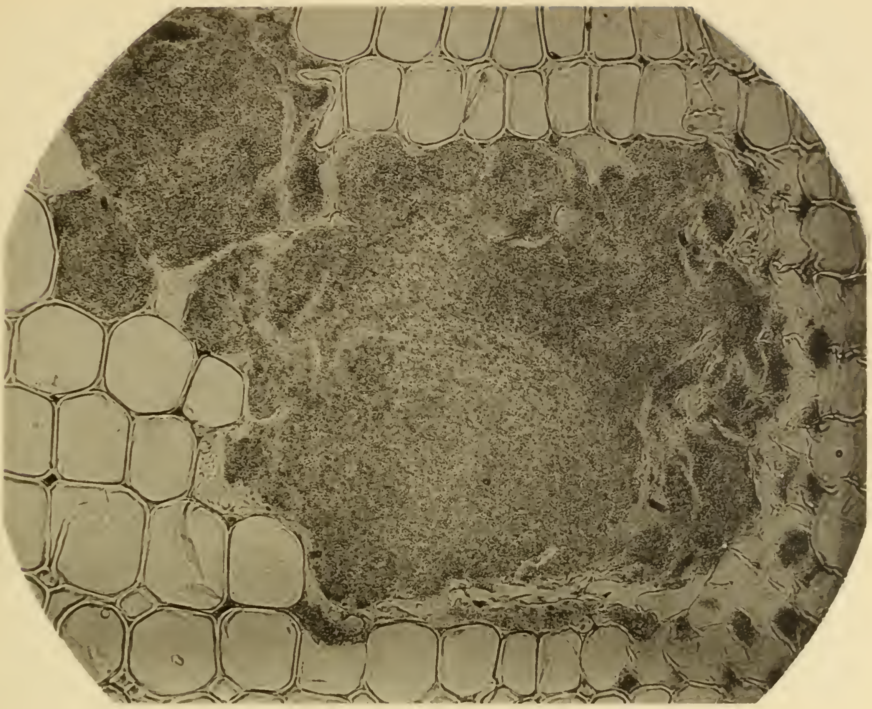

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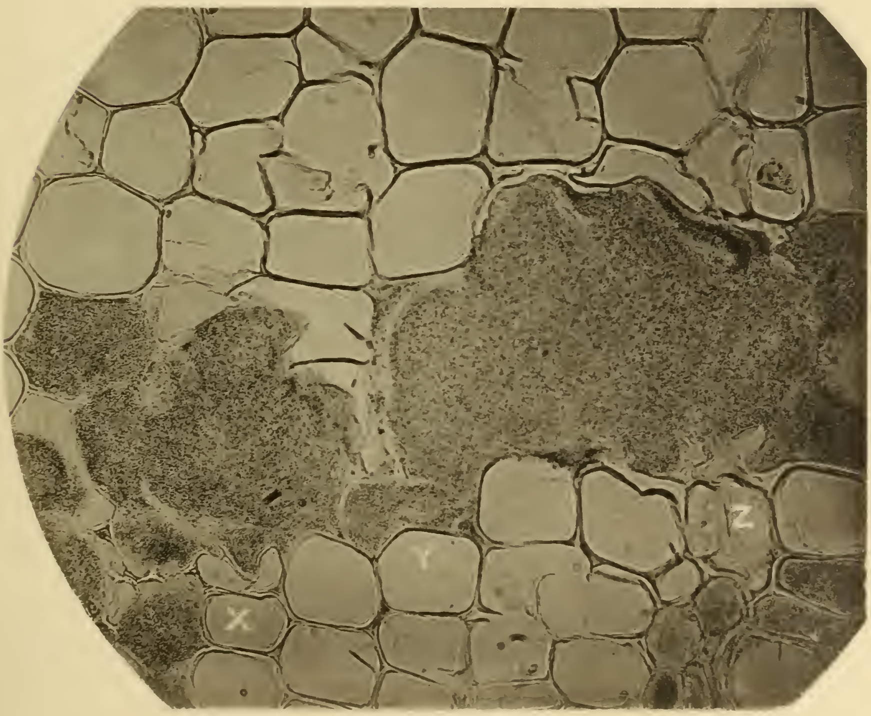

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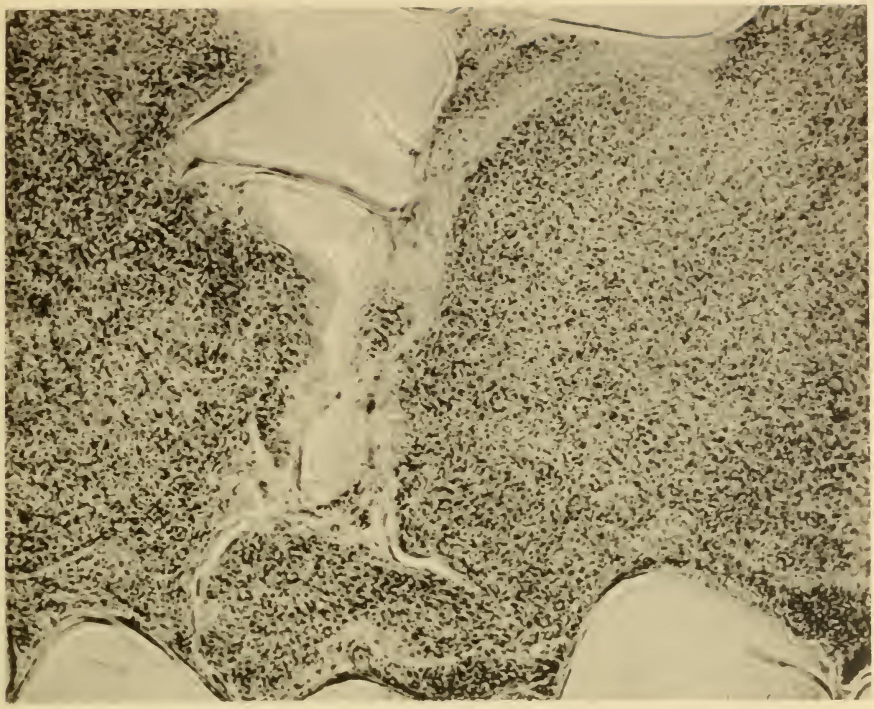

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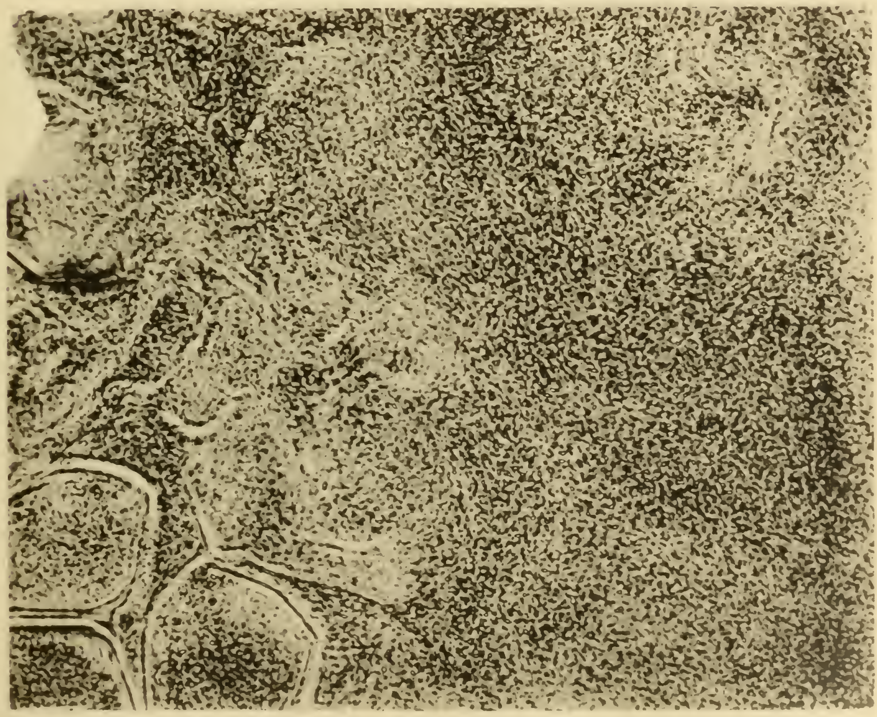

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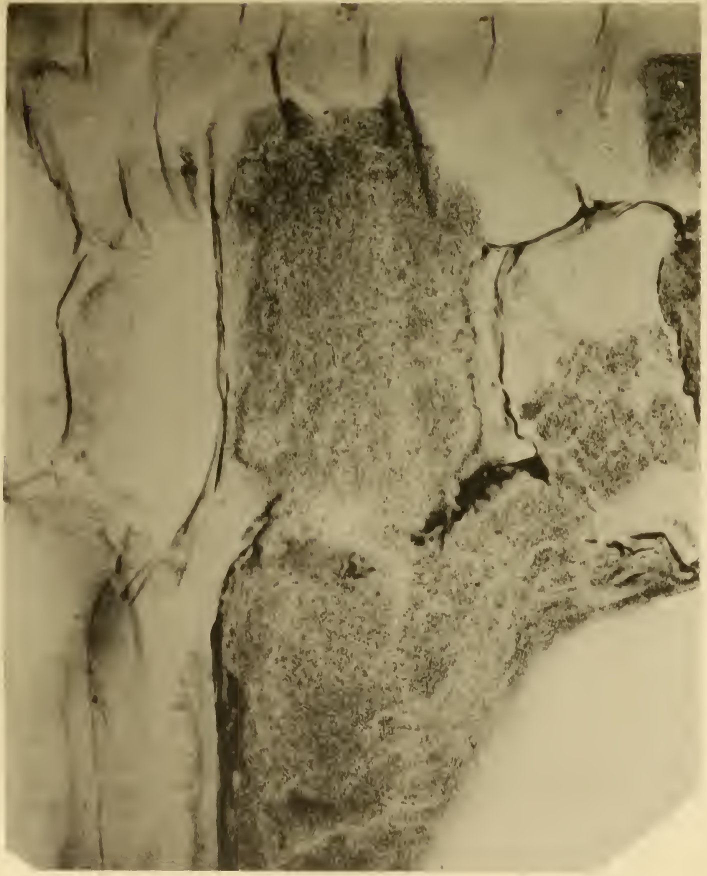

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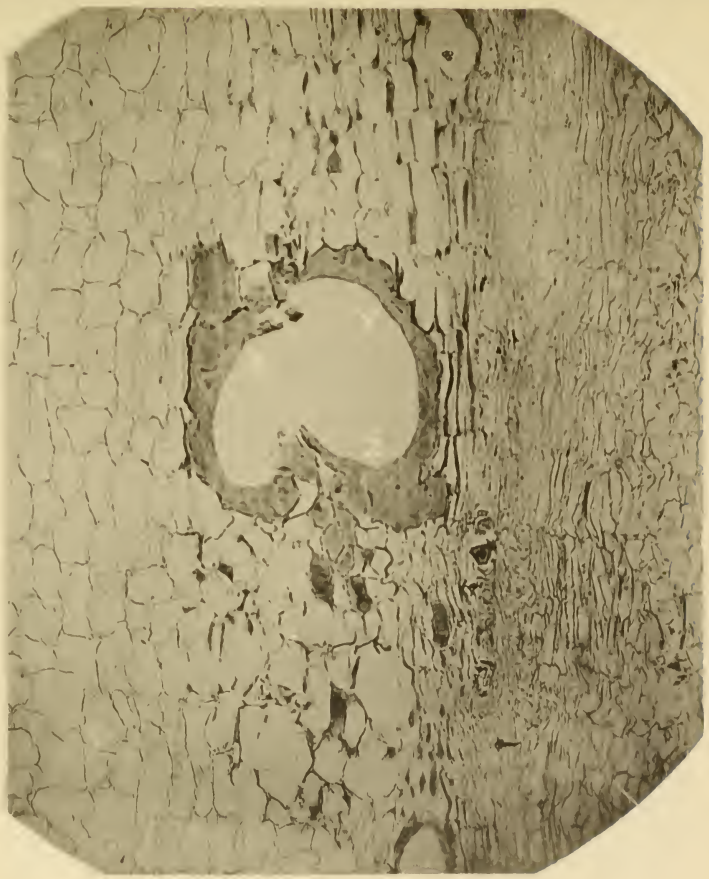

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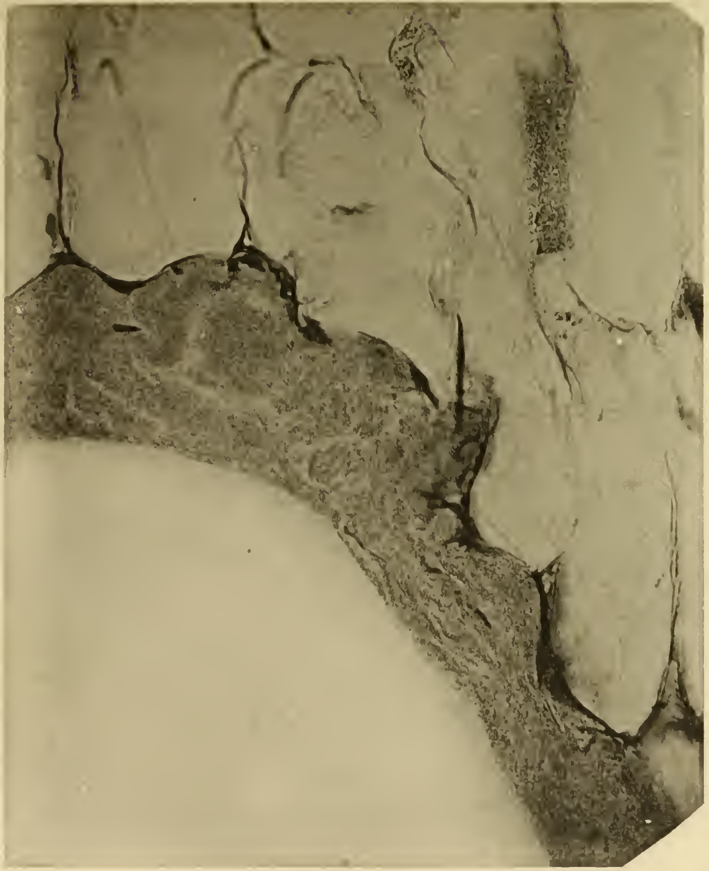

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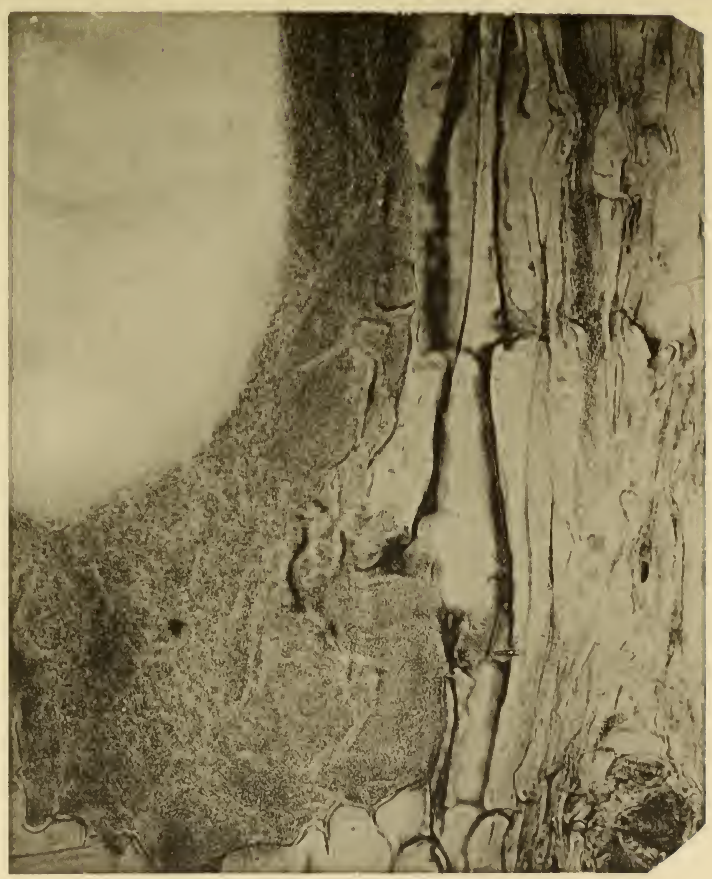

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